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Short report

Alternate day fasting with or without exercise: Effects on endothelial function and adipokines in obese humans

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SUMMARY

Objective: Alternate day fasting (ADF; which consists of an ad libitum "feed day" alternated with a 75% energy restriction "fast day") combined with exercise improves several coronary heart disease (CHD) risk factors. However, the effect of this combination therapy on endothelial function, and the role that adipokines play in mediating this effect, is unknown. Accordingly, this study examined the effect of ADF combined with exercise on brachial artery flow mediated dilation (FMD) and plasma adiponectin and leptin.

Research methods and procedures: Sixty-four obese subjects were randomized to 1 of 4 groups: 1) combination (ADF + endurance exercise), 2) ADF, 3) exercise, or 4) control, for 12 weeks.

Results: Body weight decreased ($P < 0.05$) in the combination (-6 ± 4 kg), ADF (-3 ± 1 kg) and exercise group (-1 ± 0 kg). Fat mass decreased ($P < 0.01$) in the combination (-5 ± 1 kg) and ADF (-2 ± 1 kg) groups. FMD increased ($P < 0.05$) only in the ADF group ($5 \pm 1\%$ to $10 \pm 2\%$; 5% increase). Leptin decreased in the combination (-34 ± 9 ng/ml, $P < 0.001$), ADF (-10 ± 4 ng/ml, $P < 0.05$) and exercise group (-11 ± 4 ng/ml, $P < 0.05$). Adiponectin was not changed by any intervention. Changes in FMD in the ADF group were not related to changes in leptin.

Conclusions: These findings suggest that ADF alone is an effective intervention to improve vascular endothelial function. However, the role of adipokines in mediating this effect is still unclear.

1. Introduction

Alternate day fasting (ADF) is a novel dietary restriction strategy that has gained considerable popularity over the past decade. ADF consists of an ad libitum "feed day" alternated with a 75% energy restriction "fast day". ADF regimens were created to boost adherence to dietary restriction protocols, in that they only require energy restriction every other day, instead of everyday, as with traditional calorie restriction (CR). The ability to eat freely every other day results in greater adherence to the ADF diet, when compared to CR, which has the potential to translate into greater weight loss.[1,2] Recent evidence suggests that combining ADF with endurance exercise increases HDL cholesterol levels, decreases LDL cholesterol levels, and augments both LDL and HDL particle size.[3,4] These beneficial changes in plasma lipids suggest that this combination therapy may confer protection against coronary heart disease (CHD). Endothelial dysfunction is another gold standard prognostic indicator of future CHD, and most vascular disease risk factors are associated with reduced flow mediated dilation (FMD).[5] An important question that has yet to be tested is whether the combination of ADF plus endurance exercise can elicit added cardiovascular benefits by increasing FMD.

Adipose tissue acts as endocrine organ in that it secretes biologically active hormones called adipokines. Recent evidence suggests that certain adipokines, such as adiponectin and leptin, may improve nitric oxide (NO) bioavailability and endothelial function. For instance, adiponectin induces the phosphorylation of endothelial nitric oxide synthase (eNOS), which results in increases in NO production and FMD.[6,7] Leptin, in contrast, is a pro-atherogenic

Abbreviations: ADF, alternate day fasting; CHD, coronary heart disease; METs, metabolic equivalents; HRmax, heart rate maximum; SEM, standard error of the mean; ANOVA, analysis of variance; FMD, flow mediated dilation; NO, nitric oxide; eNOS, endothelial nitric oxide synthase.

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hormone derived from adipocytes that has been shown to cause endothelial dysfunction in obese individuals.[8] Hyperleptinemia in obesity increases the production of oxidative species that scavenge NO, resulting in a decline in FMD.[8] Previous findings indicate that ADF and endurance exercise independently increase adiponectin while lowering leptin levels.[8,9] As such, combining these interventions may have an additive effect on the circulating concentrations of these adipokines, which may lead to more pronounced improvements in FMD.

Accordingly, the present study investigated the effect of ADF combined with endurance exercise on endothelial function, relative to ADF and exercise alone. The role of adipokines in mediating improvements in FMD was also investigated.

2. Materials and methods

2.1. Subjects and study design

As described previously,[4] obese subjects were recruited from the University of Illinois at Chicago by advertisements. Key inclusion criteria were as follows: age 25–65 years; body mass index between 30 and 39.9 kg/m^2; weight stable for 3 months prior to the beginning of the study (less than 5 kg weight loss or weight gain); non-diabetic; no history of cardiovascular disease; lightly active (<3 h/week of light intensity exercise at 2.5–4.0 metabolic equivalents (METs) for 3 months prior to the study); non-smoker; no history of bariatric surgery; and not taking weight loss, lipid or glucose lowering medications. The experimental protocol was approved by the Office for the Protection of Research Subjects at the University of Illinois, Chicago, and all volunteers gave their written informed consent to participate in the trial. A 12-week, randomized, controlled, parallel-arm feeding trial was implemented to test the study objectives. Subjects were stratified on the basis of BMI, age, and sex, and then randomized into 1 of 4 groups: 1) combination group; 2) ADF group; 3) exercise group; 4) control group. Randomization was performed for each stratum by selecting an intervention at random from an opaque envelope. The 12-week clinical trial was run 3 times from April 2010 through April 2011. Recruitment took place during a 4-week period before the beginning of each trial. During the second and third run of the trial, additional subjects were stratified and randomized to groups that had high dropout rates (i.e. the ADF and exercise group). This ensured that the total number of subjects would be the same in each group at the end of the study. The additional subjects did not differ with respect to demographic characteristics as compared to the original subjects.

2.2. Diet protocol

The diet intervention has been previously described.[4] Only the combination and ADF groups participated in the diet protocol. Briefly, the 12-week diet intervention consisted of two phases: 1) a controlled feeding phase (week 1–4), and 2) a self-selected feeding phase (week 5–12). During the controlled feeding phase (week 1–4) participants consumed 25% of their baseline energy needs on the "fast day" (24 h) and consumed food ad libitum on each "feed day" (24 h). All fast day meals were provided to the subjects during the controlled feeding phase. The baseline energy requirements for the subjects were assessed by the Mifflin equation.[10] Fast day meals were consumed between 12 pm and 2 pm. The macronutrient composition of the provided fast day meals was 25% kcal from fat, 20% kcal from protein, and 55% kcal from carbohydrates. During the self-selected feeding phase (week 5–12) subjects continued with the ADF regimen but no fast day food was provided to them. Instead, each subject met with a dietician at the beginning of each week to learn how to maintain the ADF regimen on his or her own at home. Control and exercise group subjects were not given any dietary counselling and maintained their regular eating habits.

2.3. Exercise protocol

Both the combination and exercise groups participated in a moderate intensity exercise intervention, 3 times/week, for 12 weeks. The supervised exercise sessions were performed at the research center using stationary bikes and elliptical machines. An age-predicted heart rate maximum (HRmax) equation [$209 - (0.7 \times \text{age})$][11] and a polar heart rate monitor (Polar USA, Inc., NY) were used to estimate exercise intensity. At the beginning of the study (weeks 1–4), each exercise session ran for 25 min duration and corresponded to 60% of the subject's HRmax. Training duration and intensity increased incrementally at week 4, 7 and 10 by 5 min and 5% HRmax. As such, by week 10, each subject was exercising for a 40 min duration at an intensity of 75% HRmax. ADF and control subjects were asked to maintain their regular activity habits, and to refrain from joining an exercise class during the study.

2.4. Body weight and body composition assessment

Body weight measurements were taken to the nearest 0.5 kg at the beginning of each week with subjects wearing light clothing and without shoes using a balance beam scale (HealthOMeter; Sunbeam Products, Boca Raton, FL, USA). Fat mass was assessed each week in triplicate using a tetra-polar bioelectrical impedance analyzer (BIA; Omron HBF-500; Omron Health Care, Bannockburn, IL, USA). Waist circumference was measured by a flexible tape to the nearest 0.1 cm, midway between the lower costal margin and super iliac crest during a period of expiration.

2.5. Brachial artery measurements of flow mediated dilation (FMD)

Brachial artery FMD was assessed at week 1 and 12. Subjects did not exercise for 24 h prior to the FMD assessment. Ultrasound imaging of the brachial artery (MicroMaxx, Sonosite, Seattle, WA) was performed in a longitudinal plane at a site 1–3 cm proximal to the antecubital fossa, with the arm abducted approximately 80° from the body and the forearm supinated. The ultrasound probe (11 MHz) was positioned to visualize the anterior and posterior lumen–intima interfaces to measure diameter or central flow velocity (pulsed Doppler). The probe site was marked for accurate repositioning after exercise. After baseline images were recorded, a blood pressure cuff on the forearm was inflated to 200 mm Hg for 5 min. To assess FMD, 10 images were captured every second. A total of 10 s of images were recorded during the process. These images were taken at 30 s, 60 s and 120 s after cuff release. Baseline brachial flow velocity and peak velocity after cuff release were recorded. Images were digitally recorded using Brachial Imager (Medical Imaging, Iowa City, IA) and analyzed. Percent FMD was calculated using the averaged minimum mean brachial artery diameter at baseline compared to the largest mean values obtained after release of the forearm occlusion. Blood pressure was assessed in triplicated after a 10-min rest.

2.6. Plasma adipokines

Twelve-hour fasting blood samples were collected between 6.00 am and 10.00 am at week 1 and week 12. Subjects were instructed to avoid exercise, alcohol and coffee for 24 h before each visit. Blood was centrifuged for 10 min at 1000 g and 4 °C to separate plasma from RBC and was stored at −80 °C until analyzed. Plasma adiponectin and leptin were measured using high sensitivity

enzymatic kits (R&D Systems, Minneapolis, MN). The intra-assay variances for the adiponectin and leptin ELISAs were 4.8 and 4.2%, respectively.

2.7. Statistical analysis

Results are presented as mean ± SEM. Differences between intervention groups at baseline were analyzed by a one-way ANOVA. When baseline differences were noted for a specific parameter, ANCOVA was performed with the baseline value as a covariate. Within-group differences were analyzed using repeated-measures ANOVA. An intention-to-treat analysis was performed for all variables measured. A P-value of <0.05 was used as a criterion for statistical significance in all analyses. Data were analyzed using SPSS software (version 20.0 for Mac OSX; SPSS Inc, Chicago, IL, USA).

3. Results

3.1. Baseline characteristics and dropouts

Eighty-three subjects began the clinical trial (combination: $n = 18$, ADF: $n = 25$, exercise: $n = 24$, control: $n = 16$), and $n = 16$ subjects finished in each intervention group (total $n = 64$). Additional subjects were randomized to groups that had high dropout rates (i.e. the ADF and exercise group) to ensure that the total number of subjects would be the same in each group at the end of the study. At baseline, there were no differences in age (combination: 45 ± 5 y, ADF: 42 ± 2 y, exercise: 42 ± 2 y, control: 49 ± 2 y), sex (combination (F/M): 18/0, ADF: 24/1, exercise: 23/1, control: 15/1), and BMI (combination: 35 ± 1 kg/m^2, ADF: 35 ± 1 kg/m^2, exercise: 35 ± 1 kg/m^2, control: 35 ± 1 kg/m^2) between groups. Moreover, there were no differences at baseline between groups for body weight, fat mass, waist circumference, and adipokines (Table 1). Systolic and diastolic blood pressures were higher in the ADF group at baseline versus the other groups. Exercise attendance remained high throughout the study in both the combination (95 ± 2% of sessions), and exercise group (94 ± 1% of sessions).

3.2. Body weight and body composition

Changes in body weight, fat mass and waist circumference are reported in Table 1. Body weight significantly decreased ($P < 0.05$) in all three intervention groups. The combination group showed the greatest ($P < 0.001$) weight loss (6 ± 4 kg), followed by the ADF (3 ± 1 kg) and exercise group (1 ± 0 kg). Fat mass decreased ($P < 0.01$) only in the combination (5 ± 1 kg) and ADF group (2 ± 1 kg) after 12 weeks. The decrease in waist circumference was higher ($P < 0.001$) in the combination group (8 ± 1 cm) compared to the ADF (5 ± 1 cm) and exercise group (3 ± 1 cm).

3.3. Brachial artery flow mediated dilation (FMD)

There were no differences in FMD between groups at baseline (Fig. 1). By week 12, FMD improved ($P < 0.05$) in the ADF group relative to baseline (5 ± 1% to 10 ± 2%; 5% increase). There were no changes in FMD in the combination, exercise or control groups over the course of the trial. Systolic and diastolic blood pressure decreased ($P < 0.05$) only in the ADF group (Table 1).

3.4. Plasma adipokines

Adiponectin levels were not affected by any of the interventions after 12 weeks of treatment (Table 1). Leptin concentrations

Table 1
Body weight, blood pressure, and adipokines during the 12-week trial.

	Intervention	Week 1	Week 12	P-value[a]	P-value[b]	Change[c]	P-value[d]
Body weight (kg)	Combination	91 ± 6	85 ± 6	<0.001	0.393	−6 ± 4[a]	<0.001
	ADF	94 ± 3	91 ± 3	<0.001		−3 ± 1[b]	
	Exercise	93 ± 2	92 ± 2	0.027		−1 ± 0[b]	
	Control	93 ± 5	93 ± 5	0.577		0 ± 0[c]	
Fat mass (kg)	Combination	45 ± 2	40 ± 2	<0.001	0.054	−5 ± 1[a]	<0.001
	ADF	43 ± 2	41 ± 2	0.008		−2 ± 1[b]	
	Exercise	46 ± 2	45 ± 2	0.182		−1 ± 0[b]	
	Control	43 ± 4	43 ± 4	0.570		0 ± 1[b]	
Waist circumference (cm)	Combination	96 ± 2	88 ± 1	<0.001	0.310	−8 ± 1[a]	<0.001
	ADF	100 ± 2	95 ± 2	<0.001		−5 ± 1[b]	
	Exercise	98 ± 2	95 ± 2	<0.001		−4 ± 1[b]	
	Control	98 ± 3	97 ± 2	0.640		−1 ± 1[b]	
Systolic BP (mm Hg)[e]	Combination	113 ± 3	111 ± 3	0.262	0.176	−2 ± 2	0.254
	ADF	124 ± 3	120 ± 3	0.007		−3 ± 1	
	Exercise	113 ± 2	115 ± 3	0.284		2 ± 2	
	Control	122 ± 5	120 ± 6	0.603		−2 ± 3	
Diastolic BP (mm Hg)[e]	Combination	76 ± 2	76 ± 2	0.939	0.123	0 ± 3	0.570
	ADF	82 ± 2	80 ± 2	0.034		−2 ± 2	
	Exercise	76 ± 2	76 ± 2	0.976		0 ± 2	
	Control	86 ± 2	84 ± 4	0.480		−2 ± 3	
Adiponectin (ng/ml)	Combination	11,452 ± 1324	12,084 ± 1470	0.369	0.101	632 ± 682	0.190
	ADF	9069 ± 1019	8487 ± 921	0.145		−582 ± 400	
	Exercise	9111 ± 1024	9813 ± 1196	0.221		702 ± 461	
	Control	8234 ± 723	8943 ± 867	0.725		709 ± 417	
Leptin (ng/ml)	Combination	63 ± 11	29 ± 7[a]	0.001	0.001	−34 ± 9[a]	0.015
	ADF	41 ± 7	31 ± 6[a]	0.028		−10 ± 4[b]	
	Exercise	44 ± 9	33 ± 6[a]	0.025		−11 ± 4[b]	
	Control	69 ± 8	73 ± 14[b]	0.760		4 ± 12[c]	

Values reported as mean ± SEM. Intention to treat analysis. ADF: Alternate day fasting, BP: Blood pressure.
[a] P-value between week 1 and week 12: Repeated-measures ANOVA.
[b] P-value between groups at week 12: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey test).
[c] Absolute change between week 1 and week 12 values.
[d] P-value between groups for absolute change: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey test).
[e] ANCOVA was performed with baseline values as a covariate.

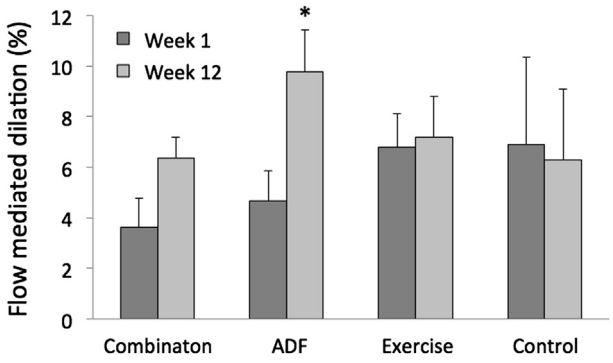

Fig. 1. Flow mediated dilation (FMD) at baseline and post-treatment. Values reported as mean ± SEM. ADF: Alternate day fasting. *Week 1 value significantly different ($P < 0.05$) from week 12 value (Repeated-measures ANOVA).

decreased ($P < 0.05$) by 34 ± 9 ng/ml, 10 ± 4 ng/ml, and 11 ± 4 ng/ml in the combination, ADF, and exercise group, respectively. There were no associations between FMD and adipokine concentrations at baseline or post-treatment in any intervention group.

4. Discussion

This study is the first to show that ADF is an effective intervention to improve endothelium-dependent flow mediated dilation. The improvements in FMD by ADF were not mediated by changes in adiponectin or leptin, however. Interestingly, exercise alone or in combination with ADF, had no effect on FMD.

In the present trial FMD increased in the ADF group only. Leptin levels were also decreased by the ADF intervention, however, no relationship was noted between lower leptin levels and increased FMD. It is possible that the correlation between leptin and FMD was not significant due to the small sample size of the ADF group. Contrary to previous reports,[12,13] adiponectin was not altered by 12 weeks of ADF. Seeing as adiponectin is only increased with substantial weight loss (>10% from baseline), the weight loss achieved here may not have been sufficient to augment levels of this adipokine.[14] It is possible that FMD only improved in the ADF group because this was the only intervention to demonstrate decreases in blood pressure. In a review by Dharmashankar et al, the association between hypertension and endothelial dysfunction was assessed.[15] Findings from this review indicate that high blood pressure increases oxidative stress and inflammation resulting in endothelium-dependent vasomotor dysfunction.[15] Therefore, the change from the pre-hypertensive to normotensive state in the ADF group may have reduced the oxidative stress and inflammation, thus improving FMD. Since blood pressure was not altered by the exercise or combination intervention, this may explain why FMD did not increase in either of these groups. The physiological reason for this lack of effect remains to be determined.

Our study is limited in that a wide age range (25–65 years) was implemented. Celermajer et al. has shown that endothelial dysfunction increases with aging in both men and women.[16] Therefore, this factor should be considered when interpreting the data.

As for endurance exercise, no change in FMD was observed after 12 weeks of treatment, despite reductions in leptin concentrations in this group. It is possible that the exercise intensity in the current study (60%–75% HRmax) was not high enough to increase FMD. In a study by Tjonna et al, the effect of high intensity (90–95% HRmax) versus moderate intensity (75% HRmax) aerobic exercise on FMD was examined.[17] FMD improved to a greater extent in the high intensity group as compared to the moderate intensity group. The researchers speculated that the high intensity exercise produced greater sheer stress on the blood vessel walls, thus producing positive molecular changes.[17] Therefore, it is possible that a higher intensity of exercise (>90% HRmax) is required to improve endothelial function. Furthermore, a higher caloric expenditure with endurance exercise, which would result in greater weight loss, may also be required to improve endothelial function.[18] These factors may explain why no changes in FMD were observed after 12 weeks of endurance training.

In summary, these findings suggest that ADF is an effective strategy to improve endothelium-dependent flow mediated dilation. However, the role of adipokines in mediating this effect is still not clear. We also show here that a moderate intensity exercise regimen may not be sufficient enough to improve FMD, thus explaining why the combination and exercise group demonstrated no change in this CHD risk parameter. Overall, this study contributes to the mounting evidence supporting role of ADF in the prevention of CHD in obese populations.

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Conflicts of interest

The authors have no conflicts of interest to report.

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SB designed the study, conducted the clinical trial, analyzed the data, and wrote the manuscript. MCK and CMK assisted with clinical trial coordination. SAP, EN, JFT assisted with the data analysis and wrote the manuscript. KAV designed the study and wrote the manuscript.

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